THE ABSORPTION OF FAT BY FRESHWATER MUSSELS.[1]

E. P. CHURCHILL, JR.

INTRODUCTION.

The present paper embodies the results of the first of a series of investigations designed to ascertain whether or not animals living in the water use food which is in solution in the surrounding medium in addition to "formed" food such as plankton, etc. If it is found that such food in solution is used, it is of importance to learn whether it is absorbed by the alimentary tract alone or to some extent by the epithelium of the outer body walls, especially that of the gills in the case of mussels.

Pütter ('08) was the first to advance the theory that food could be so taken. He based his conclusions in part on a comparison of the amount of carbon necessary for the maintenance of the organism with the amount furnished by the plankton. Pütter considered the latter too small for the needs of the animal and argued that it must use some carbon which is in solution in the water, resulting from the decay and disintegration of organic life. He further stated that the amount of material found in the alimentary canal is never large enough to supply the requisite quantity of carbon. Besides the alimentary canal, the uncutinized epithelium of the outer surface of the body, especially that of the gills, was thought to function in absorbing dissolved food. This process went on in addition of course to the digestion of "formed" food by the alimentary canal. Pütter concluded that the above conceptions applied to Protozoa, Porifera, Echinoderms, Crustaceans, Mollusks and Fishes. He tested the matter experimentally in two ways: first by noting that goldfish and perch lived much longer in solutions of asparagin, somatose and glycerin than in tap water: secondly by com-

[1] Contribution from the United States Biological Station, Fairport, Iowa. (Two of the experiments were carried out at the zoölogical laboratory of the Johns Hopkins University.) Published by permission of the Commissioner of Fisheries.

paring the amount of oxygen needed to oxidize the lost weight of tissues of actinians, tunicates and fish while kept in their natural medium with the amount of oxygen actually used. As the latter was found to be greater than the estimated quantity needed he concluded that the extra oxygen was used in oxidizing some food that had been taken from the water where it was present in the form of a solute.

Knorrich (10), working with *Daphnia*, which lived 14 days in sterilized hay solution, concluded that nutriment was absorbed from the solution.

Kerb (10) kept eels in sugar solution and noted no diminution of the amount of sugar from day to day. He obtained similar results while working with *Corethra* larvæ in sugar solutions. Also he found that *Daphnia* lost in dry body weight as rapidly in solutions of peptones as in tap water.

Wolff (10), working with *Simocephalus*, found that it lived twice as long in bacteria-free water, which contained some dissolved carbon compounds, as it did in tap water. He made no observations as to body weight lost or gained.

Lipschütz (13) reviewed the entire subject including his own previously published experiments along that line and offered criticism of Pütter's work. Lipschütz noted that fish and eels when kept in nutrient solutions lost as much weight as in tap water. He also thought that Pütter overestimated the amount of material in solution in the water and underestimated the carbon content of the plankton. His general conclusions are the opposite of those of Pütter.

Lund[1] found that if Protozoa are kept in a weak soap solution they will absorb fat from such solution through their body walls. At his suggestion the fats used in the following experiments were rendered soluble in water by saponification.

I am especially indebted to Dr. Caswell Grave, at whose suggestion the work was undertaken, for his advice and aid on many occasions and his supervision of the preparation of the manuscript. I also desire to express my obligations to Dr. E. J. Lund for suggestions concerning some of the chemical reactions

[1] Dr. Lund's paper is not yet published, his results having been communicated to me verbally.

discussed in the latter part of this paper; to Dr. G. L. Houser for the use of several books and a microscope from the laboratories of the University of Iowa; and to Dr. R. E. Coker for aid rendered while the work was being carried on at the Fairport Laboratory.

Materials and Methods.

The mussels upon which the investigations were carried out were individuals selected from the more common species found in the Mississippi River near Fairport, Iowa. Both adult and juvenile specimens were employed. Care was exercised to choose for the experiments non-gravid mussels which were in a seemingly healthy condition, "shoulder-raked" or hand collected individuals being generally used in preference to those dragged out of the water by "crow-foot" hooks.

The water used in the experiments was that of the general supply for the Fairport Laboratory which had come from the Mississippi River through a filter which removed the mud and at least the greater part of the living animal and plant organisms.

The fats employed were those of olive oil and cottonseed oil. The results were the same in both cases as far as could be observed. The oils were saponified by boiling with sodium hydroxide, the resulting solution being diluted to strengths varying from .001 per cent. to .005 per cent. in different cases. This method can be used only with freshwater animals as the soap is precipitated from the solution in salt water.

The adult mussels experimented with were kept in glass aquaria containing about 5,000 c.c. of the fat solution of the desired strength. The bottoms of the aquaria were covered to a depth of an inch or two with coarse sand. Control individuals were run side by side with the experiments in similar aquaria containing filtered water only. The juvenile mussels were generally kept in smaller aquaria. In the cases of experiments extending over more than twenty-four hours, the solutions were changed daily, no effort being made to balance the aquaria. As the mussels from the river are accustomed to a current of water this seemed to be the closest approximation to natural conditions which it was possible to obtain in the laboratory.

The tissues of various portions of the adult animals experi-

mented with and of the control mussels were sectioned by the freezing method, stained with Sudan III. and mounted in glycerin. Also sections of the tissues of the adult and of the entire bodies of the juvenile mussels were made after fixation in osmic acid and Muller's fixing fluid. In this method the higher alcohols were avoided by clearing the sections in clove oil from 85 per cent. or 90 per cent. alcohol and infiltrating in paraffin from the clove oil. The paraffin was removed with xylol and the sections were mounted in xylol balsam. Such sections were studied very shortly after mounting, as the fat is gradually dissolved out by the xylol.

In some cases the fat solution was stained by dissolving Sudan III. in the same to the point of saturation. The external appearance of various parts of mussels which had been kept in such stained solutions was noted. Also frozen sections of such mussels were examined to ascertain whether or not stained fat had been absorbed from the solution. In such cases the sections were mounted in glycerin directly after cutting.

Observations.

The mussels were found to live readily in .001 per cent. to .005 per cent. fat solutions. The only abnormal manifestation to be observed was that a considerable quantity of mucus was thrown off. Six adult individuals which were kept in fat solution for one month were living at the close of that period. At the expiration of the same length of time one of the control mussels of this experiment had died. After the adoption of the method of manipulating the aquaria outlined above, no mussels died while kept in fat solutions varying in strength from .001 per cent. to .005 per cent. However, beyond the fact that mussels can live for at least one month in fat solutions of these strengths, no evidence was secured concerning the relative longevity of those kept in fat solutions and of the control individuals. No weights were taken. Experiments calculated to ascertain whether or not the absorption of fat from the solutions employed is of advantage to mussels were deferred until the actual entrance of the fat into the tissues of the animal was proved. Emphasis was therefore laid upon the histological evidence of the absorption

of fat and it is such evidence that this paper is designed to set forth.

Mussels which had been kept in unstained fat solution will be considered first. Camera lucida drawings were made of typical portions of the sections, care being taken to indicate as far as possible the number and position of the fat droplets.[1] In some cases the fat was so massed together that individual droplets were indistinguishable. Fat is represented in all the drawings by heavy black dots or areas.

The case of an adult specimen of *Quadrula ebena* which had been kept in .001 per cent. fat solution for 15 days will first be discussed. Sections of the gill filaments of this mussel were prepared after fixation in a solution of osmic acid and Muller's fixative. In such sections an abundance of fat droplets was to be seen in the epithelial cells and a number in corpuscles in the blood vessel in the interior of each filament (Fig. 1, Pl. I.). Very few fat droplets were found in sections of the control individual prepared by the same method (Fig. 2, Pl. I.). Frozen sections, after having been stained with Sudan III., gave results parallel to those just stated—a large number of fat droplets in the gill filaments of the mussels which had been kept in fat solution for 15 days (Fig. 3, Pl. I.), but practically nothing that took the Sudan III. stain in the control individuals. The epithelial lining of the water tubes of the gills of the individuals which had been kept in fat solution was also quite crowded with fat. In sections of the intestine of mussels which had been kept in fat solution, fat droplets appeared in the epithelial lining. Also in sections of palps and mantle fat droplets were revealed but not in such abundance as was true in the case of the gills. Fat droplets were quite numerous in the cells of the side of the mantle next to the body but there were practically none in the cells of the side lining the shell. The tissues of three adult mussels which had been kept in fat solution for 15 days and of two control individuals which had remained in filtered water for the same period were examined by the two methods of preparing

[1] The term "droplets" will in this paper be applied to the spherules of fat found in the tissues of the mussels which were studied. These droplets usually were of diameters varying from 5 to 10 microns, though in frozen sections of the gills instances were found in which the diameter was as great as 20 microns.

sections. The former all presented abundant evidence of the presence of fat. The difference between those which had been kept in the solutions and the control mussels was as striking as that shown in Figs. 1 and 2.

The best results were obtained from the study of juvenile mussels as in these cases the entire animal could be sectioned serially and the distribution of the absorbed fat over the entire body observed. A specimen of *Anodonta imbecillis* which had been kept in .002 per cent. fat solution for 10 days and a control specimen of the same species which had been kept in filtered water for the same length of time were selected as typical cases for study.

Figs. 4 and 5, Pl. II., showing corresponding parts of the foot, give an idea of the abundance and distribution of fat in the mussel which had been kept in the fat solution and of the almost entire absence of it in the case of the control specimen. (In this case as well as in that of several of the other figures, no effort has been made to draw the outlines of the epithelial cells, the intention being merely to represent the relative amount and distribution of the fat.) It will be noted that the fat is very abundant in the epithelium. It is present in some quantity in the interior of the folds of the foot and also within the deeper parts of the foot, apparently adhering to muscle fibers. The fat droplets exhibit some tendency here, and more markedly elsewhere, to gather together in clumps or to form chains. Several blood corpuscles which contain fat may be seen.

Fig. 6, Pl. II., shows a portion of the foot of the same individual, extending from the base of a fold to the blood vessel in the center of the foot. An abundance of fat was observable in the epithelium and quite numerous droplets appeared clinging to muscle fibers or enclosed within corpuscles in the central part of the foot.

Fig. 7, Pl. II., representing a portion of a fold of the foot, was drawn under the 1/12 oil immersion lens, an effort being made to depict the exact number and position and also the relative size of the fat droplets, muscle fibers and cells.

Fig. 8, Pl. I., shows in (*a*) the cross section of a liver tubule of the mussel used as the control, the few coarse stipples represent-

ing the fat present; and in (*b*) the cross section of the corresponding part of the mussel which had been kept in the fat solution. In the latter the liver cells are seen to be heavily loaded with fat, in some cases practically filled with it. A very great abundance of fat was found throughout the liver cells in all sections of this mussel.

Figs. 9, 10, and 11, Pl. I., are drawings made from the sections of the epithelial cells, respectively, of the intestine, of a gill filament and of the side of the mantle next to the body, of the specimen of *A. imbecillis* which had been kept in the fat solution for 10 days. They represent as accurately as could be drawn with the camera lucida the relative amount and arrangement of the fat droplets. Many corpuscles containing fat were found in the blood lacunæ immediately outside of the cells of the intestine. Some of these corpuscles were lying at the bases of the cells as shown in Fig. 9. Corpuscles containing fat were also found in nearly all blood vessels of the gill filaments. Many such corpuscles were in the position shown in Fig. 10, that is, close against the bases of the cells of the filament. In the mantle fewer corpuscles were seen. Here the fat was found adhering to muscle fibers and strands of connective tissue.

Figs. 12 and 13, Pl. III., represent mesenchyme cells and blood corpuscles of the mussel which had been kept in the fat solution and of the control individual respectively. In these sections may be observed the relation of the fat droplets to the tissues of the areas occupied by mesenchyme cells, which are especially numerous in the dorsal part of the body of the mussel. Fat was found scattered quite thickly throughout such regions in the case of the animal which had been in the solution.

The gill of an adult specimen of *Quadrula ebena* was sectioned immediately after it had been taken from the river. Fig. 14, Pl. I., represents the relative amount of fat found in its gill filaments. Somewhat more fat appeared in these sections than in those prepared from the tissues of the control mussels which had been kept in filtered water for 10 or 15 days but a very much smaller quantity was found than in the mussels which had been kept in fat solution.

The mussels which had been kept in the fat solution that had

been stained with Sudan III. will now be considered. Before beginning the experiment the valves of the shell were opened sufficiently to permit the observation to be made that the tissues of the animal to be experimented with were of normal color and condition. The mussels were then kept in stained fat solution for periods varying from 5 to 15 days. At the expiration of these periods the gills of the adult and the gills, mantles and foot of the juvenile animals were found to be red or pink in color. Sections of the gills of such adult mussels were prepared by the freezing method. In these sections pink colored fat droplets were found within the epithelial cells of the gill filaments and of the water tubes (Fig. 15, Pl. I.). This fact furnishes additional proof of the absorption of fat from the solution. It renders negligible the consideration that the heavy loading of fat found in each case in the mussels which had been kept in the fat solutions might have been due to the chance use of an extraordinarily fat individual. Some of the juvenile mussels which had taken on a red color while they had been kept in the stained fat solution were then transferred to filtered water. The color remained visible in the foot and gills for more than a week in some cases. This would tend to show that the red colored fat was absorbed and oxidized within the organism and that the red color was not due merely to the adherence of the stained solution to the surface of the gill or foot.

In all, including those mussels which were kept in stained and unstained fat solutions and the control individuals, the tissues of twelve mussels were examined. The sections all revealed much fat within the tissues of the mussels which had been kept in the fat solutions and very little or none in those of the control individuals. Consideration of the above results leaves no doubt of the fact that fat may pass from a fat solution into the body of the mussel. Now there remains the question of whether it is all absorbed through the intestine or partly through the tissues of the outer body walls. Experiments intended to throw light upon this question were undertaken with juvenile mussels. These had been kept in water containing little food for several weeks so that their tissues contained little stored fat at the beginning of the experiment.

Four mussels were kept in a .005 per cent. fat solution for the several periods of 4, 7, 18 and 24 hours. A small amount of fat was found in the epithelium of the intestine, mantle, gills and foot of the individual of the 4-hour period. None was found in the other parts of the body. About the same amount and distribution of fat was observable in the case of the mussel which had been in the solution for 7 hours. In the animal which was kept in the fat solution for 18 hours a very appreciable quantity of fat was found in regions corresponding to those in which it had been found in the mussels which had remained in the solution for 4 and 7 hours. A small amount was noted in the tissues immediately beneath the epithelium. None was found in the deeper body tissues, a fact which is in striking contrast to the case of the mussel which had been kept in fat solution for 10 days. The mussel which had remained in fat solution for 24 hours contained a considerable amount of fat in the same parts of the body in which it was found in the cases of the individuals which had remained in the solution for 4, 7, and 18 hours. Even more fat was found in the epithelium of the gills, mantle and foot than in that of the intestine (Figs. 16, 17, 18, 19 and 20, Pl. III.). A very small quantity of fat was observable in the liver cells. In certain parts of the foot a moderate amount of fat appeared immediately beneath the epithelium, some of which fat was adhering to the muscle fibers. The fat droplets became progressively less numerous as the tissues were studied to a greater depth within the foot and none were found among the muscle fibers or in the other tissues making up the main central mass of the foot (Fig. 17, Pl. III.). The corpuscles, especially those found in the gills, contained fat in many cases. In the gills the crowding of the corpuscles against the bases of the cells of the filaments was marked (Figs. 18 and 19). Every appearance was given that the fat was absorbed from the solution by the epithelial cells of the gills and was taken up from them by the corpuscles in some cases and in others thrown directly into the plasma of the blood. Fig. 20, Pl. III., shows a portion of the epithelium of the mantle. Fat was seen in the epithelial cells of the side next to the body and for a short distance beneath them but it was not found scattered throughout the deeper parts

of the mantle. No fat was found in the cells of the side of the mantle next to the shell.

The observations made on the mussels which were kept in fat solution for the periods of 4, 7, 18 and 24 hours may be summarized as follows:

1. More fat was found in the epithelium of the mantle, gills and foot than in that of the intestine.

2. In the case of the mussels which were kept in fat solution for 4 and 7 hours, fat was found only in the various epithelia.

3. In the mussel which remained in fat solution for 18 hours, much more fat than in the two above cases was found in the epithelium and a small amount was observable at various points beneath the epithelium, but none could be discerned distributed throughout the deeper body tissues.

4. In the mussel which had remained in fat solution for 24 hours, a very marked quantity of fat was found in the epithelium and a considerable amount appeared beneath the epithelium, diminishing in quantity, however, toward the interior of the body so that there was none distributed throughout the deeper body tissues.

5. No fat could be seen in the epithelium of the side of the mantle next to the shell but an abundance was present on the side which had been in contact with the solution.

6. Scarcely any fat was found in the cells of the liver.

From these facts it is probable that fat is absorbed by the outer epithelium of the body as well as by that of the intestine. Unless such were the case it would be very difficult to account for the presence of as much fat in the epithelium of the gills, mantle and foot as in that of the intestine, taking into consideration the fact that the mussels had been kept in the fat solution for such short periods, viz., intervals varying from 4 to 24 hours. It would also be difficult to account for the fact that such a very small amount of fat appeared within the deeper body tissues of these mussels while a very marked quantity was found in the epithelium of the outer body walls. It is very unlikely that the blood carried such a large amount of fat from the intestine to the outer epithelium in such a short time and during the same time carried none to the muscle fibers, mesenchyme cells or

connective tissue or conveyed none to the liver, which is the normal fat storing organ. If all the fat were absorbed by the intestine it seems certain that the sections of one at least of the mussels which had been kept in the fat solution for the short periods would have revealed more fat within the deeper body tissues than was found there. Wherever fat was found beneath the outer epithelium of these mussels which had been in the solutions for the short periods, much the larger quantity lay in close proximity to that epithelium and the amount lessened to zero toward the interior of the body. While the quantity of fat beneath the epithelium increased with the length of the time the mussel was kept in the solution, in all cases it was greatest nearer the epithelium. Apparently the fat found lying closely beneath the outer epithelium of the body had been absorbed and passed into the deeper tissues by that epithelium and had not come from the intestine. Also, if all the fat were absorbed by the intestine and the blood did transport it almost entirely to the outer epithelium it would seem most likely that the cells of the side of the mantle next to the shell would receive at least an observable amount.

In order further to test the question of whether or not fat may be absorbed from a fat solution by the epithelial cells of the outer body walls of the mussel the following experiment was performed. The valves of three adult mussels were wedged open with bits of wood and the animals suspended on a wire rack over the fat solution so that only the ventral parts of the mantle and foot were immersed. The mouth and siphons were above the solution so that it was not likely that any of the solution could enter the intestine by way of the oral or anal openings. One mussel was thus treated for 6 hours, the other two for 22 hours. They were compared with a fourth individual which had not been in the solution, all four mussels having been removed from the river only four or five days previous to the experiment. One of the animals which had been treated as above for 22 hours did not have much fat throughout the deeper body tissues but a moderate amount appeared in the epithelium of the part of the mantle which had been immersed in the solution. The other mussel which had been treated for 22 hours contained some

fat throughout the body and a heavy amount in the epithelium of the part of the mantle which had been exposed to the solution (Fig. 21*a*, Pl. III.). The control individual for this experiment contained much more fat throughout the deeper body tissues than the other three individuals but very little in the epithelium of the ventral part of the mantle (Fig. 21*b*, Pl. III.). The ventral part of the foot of the mussel which had been so treated for 6 hours revealed a moderate amount of fat in the epithelium but scarcely any in other parts of the foot. The fat found throughout the deeper body tissues of these individuals was of course previously stored fat, the mussels, as stated above, having been removed from the river but a short time. The point intended to be made is that a heavier loading of fat is found in the epithelium which had been in contact with the fat solution than in that which had not. While there is a possibility that some solution may have been carried by ciliary action up the mantle to the mouth, this probably did not occur as, in sections of the intestine, absolutely no fat was found in the epithelial cells. It seems highly probable that the epithelium of the mantle and foot absorbed the fat directly from the solution.

In regard to the mechanism of absorption of the fat by either the intestine or body walls little can be offered. The matter is quite complicated, some of the same possibilities entering here that serve to render the method of absorption of fat by the mammalian intestine as yet obscure. In some of the sections, especially in those cut by the freezing method, numerous droplets which took the stains used were found closely attached to the outer ends of the epithelial cells of the gills or mantle. These droplets were probably the fatty acids, a small part of which were present in the soap solution due to hydrolysis, by which process sodium hydroxide and the fatty acids would be formed; the remaining droplets were no doubt due to a slight acidity of the surface of the living cells resulting from the union of carbon dioxide from the cells with the water, forming carbonic acid. The fat may have been taken into the cells from these droplets upon the surface by phagocytic action or by solution in the plasma membrane, or it may have come directly from the sodium soap, the collection of the droplets upon the surface of the cells

being a phenomenon extraneous to the process of absorption. The latter interpretation seems the more probable as the droplets were not found upon the surface of the cells in nearly all the sections of tissues which had absorbed fat. The sodium may enter the cells with the acid radical and later be separated, or the fatty acid may be split off outside and enter the cell alone. As the fatty acid radical is the constituent of the fat molecule which takes the stain, only that radical could be followed in my preparations. In nearly all cases a fairly definite, though narrow, area lies between the outer ends of the cells and the fat clusters within. This band is the plasma membrane. The fact that no fat droplets could be observed actually in this membrane points to the conclusion that absorption of fat is effected by its solution in the plasma membrane and precipitation within the cell.

The manner of transportation of the fat within the body deserves mention. The bases of the cells of the intestine are in contact with blood lacunæ. Blood vessels traverse the gill filaments. A meshwork of blood lacunæ lies in the connective tissue beneath the epithelium of the mantle and foot. A considerable amount of fat is taken up and transported by the corpuscles to various parts of the body. Evidence of this is clearest in sections of the intestine and gills (Figs. 1, 9, 10, 18 and 19), in which corpuscles containing fat are found in close contact with the bases of the epithelial cells. Fat-loaded corpuscles are found in the blood spaces throughout the body of the mussel which had been kept in fat solution for several days. However, sections of the foot and mantle reveal many fat droplets lying immediately beneath the epithelium and clinging to muscle fibers or to the connective tissue instead of being contained in corpuscles (Figs. 4, 7, 11 and 17). No doubt, besides being transported by corpuscles, fat is thrown directly into the blood stream and carried thus to various parts of the body. In some cases the fat droplets may have become attached to muscle fibers, etc., as was observed in the sections, during the preparation of the tissues. In other cases the attachment may have occurred before the mussel was killed, the tissues to which the fat droplets were adhering being those which were later to oxidize the fat.

SUMMARY.

1. Fat which is in solution in water can be absorbed by fresh-water mussels.

2. Such absorption is accomplished by the epithelium of the intestine and also most probably by that of the gills, mantle and foot.

3. Fat is transported both by the blood corpuscles and by the plasma directly.

LITERATURE CITED.

Kerb.

'10 Über den Nährwert der im Wasser gelösten Stoffe. Revue d. ges. Hydrobiologie u. Hydrog., Bd. 3, 1910.

Knorrich.

'10 Studien über die Ernährungsbedingungen der Daphnien. Plöner Berichte, 1910.

Lipschütz.

'10 Zur Frage über die Ernährung der Fische. Z. f. allg. Phys., Bd. 12, 1910.

'10 Über den Hungerstoffwechsel der Fische. Ebenda.

'13 Die Ernährung der Wasserthiere durch die gelösten organischen Verbindungen der Gewässer. (Eine Kritik.) Ergebnisse der Physiologie, Bd. 13, 1913.

Putter.

'08 Die Ernährung der Wassertiere. Z. f. allg. Phys., Bd. 7, 1908.

'09 Die Ernährung der Fische. Ebenda, Bd. 9, 1909.

'11 Die Ernährung der Wassertiere durch gelöste organische Verbindungen. Pflüger's Archiv, Bd. 137, 1911.

'11 Der Stoffwechsel der Aktinien. Z. f. allg. Phys., Bd. 12, 1911.

Wolff.

'10 Ein einfacher Versuch zur Pütterschen Theorie von der Ernährung der Wasserbewohner. Intern. Revue d. ges. Hydrob. u. Hydrogr., Bd. 2, 1910.

EXPLANATIONS OF PLATES.

All drawings were made with camera lucida; Leitz Oc. No. 4, Obj. No. 6, unless otherwise indicated. The drawings are of sections prepared by the osmic acid method unless otherwise stated. Fat is represented by heavy black dots or areas.

PLATE I.

FIG. 1. Gill filament of adult *Quadrula ebena* after remaining in fat solution 15 days. *a*, epithelial cells. *b*, blood vessel. *c*, blood corpuscles. *d*, chitinous rods.

FIG. 2. Gill filament of the control for above figure: mussel had remained in filtered water 15 days. *a*, *b*, *c*, *d*, same as in Fig. 1.

FIG. 3. Gill filament of adult *Quadrula ebena* after remaining in fat solution 15 days. Frozen section, Sudan III.

FIG. 8. *a*, Cross section of liver tubule of juvenile *A. imbecillis* after remaining in filtered water 10 days, the control. *b*, Cross section of liver tubule of juvenile *A. imbecillis* after remaining in fat solution 10 days.

FIG. 9. Epithelial cells of anterior part of intestine of juv. *A. imbecillis* after remaining in fat solution 10 days. *a*, epithelial cells. *b*, blood corpuscles.

FIG. 10. Gill filament of juv. *A. imbecillis* after remaining in fat solution 10 days. *a*, blood corpuscles.

FIG. 11. Portion of epithelium on side of mantle next to the body of juv. *A. imbecillis* after remaining in fat solution 10 days.

FIG. 14. Gill filaments of adult *Quadrula ebena* sectioned immediately after taken from the river.

FIG. 15. Portion of gill filament of adult *Quadrula ebena* after remaining in Sudan stained fat solution 15 days. Black areas represent droplets of pink colored fat. Frozen section.

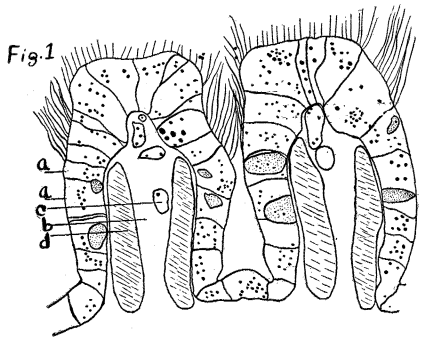


Fig. 1
a
a
c
b
d

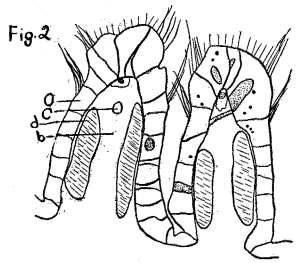


Fig. 2
a
c
d
b

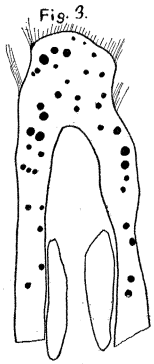


Fig. 3.

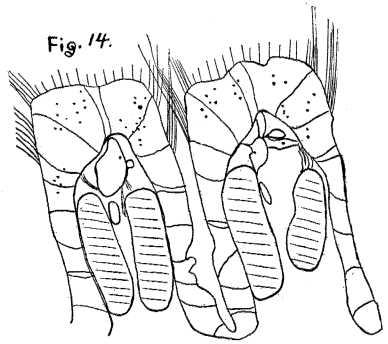


Fig. 14.

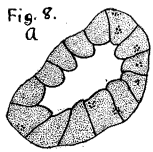
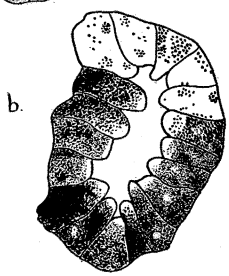


Fig. 8.
a


b.

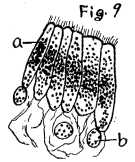


Fig. 9
a
b

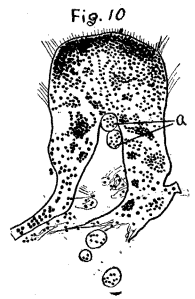


Fig. 10
a

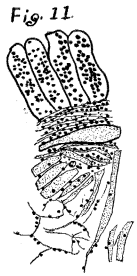


Fig. 11.

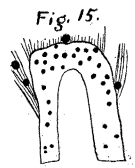


Fig. 15.

PLATE II.

FIG. 4. Portion of foot of juv. *A. imbecillis* after remaining in fat solution 10 days. Epithelial cells not indicated. Fat shown heavily massed in epithelium and adhering to muscle fibers and in corpuscles. *a*, muscle fibers in cross section. *b*, blood corpuscles. *c*, muscle fibers in longitudinal section.

FIG. 5. Portion of juv. *A. imbecillis* after remaining in filtered water 10 days. Control for Fig. 4.

FIG. 6. Portion of foot of same individual as in Fig. 4. *a*, epithelium. *b*, muscle fibers in longitudinal section. *c*, muscle fibers in cross section. *d*, blood corpuscles.

FIG. 7. Portion of fold of foot of same individual as in Fig. 4. 1/12 oil immersion. *a*, epithelial cells. *b*, muscle fibers.

Fig. 7.

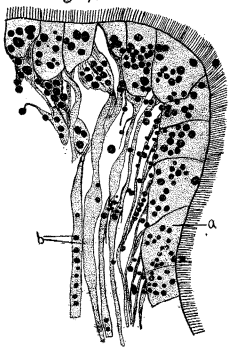


Fig. 4.

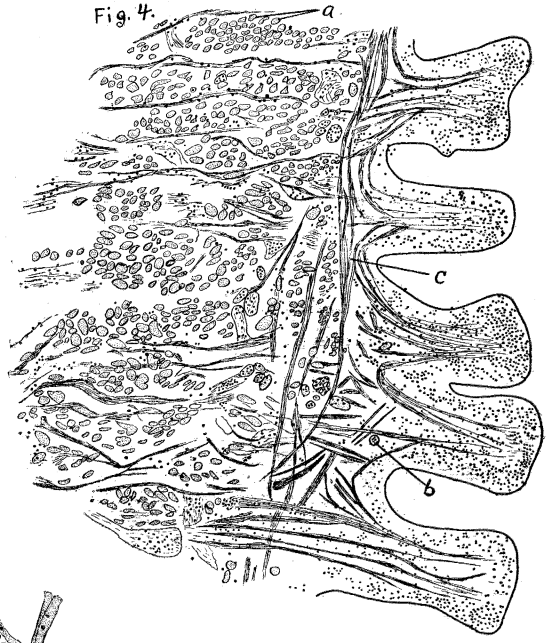


Fig. 6.

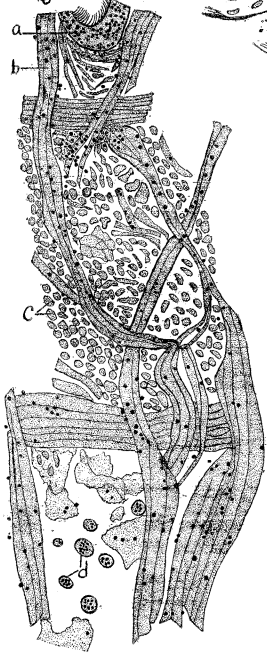


Fig. 5

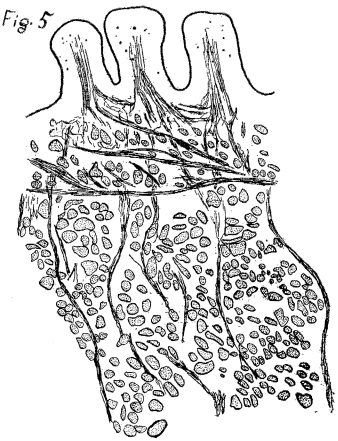


PLATE III.

FIG. 12. *a*, mesenchyme cells and *b*, blood corpuscles of juv. *A. imbecillis* after remaining in fat solution 10 days.

FIG. 13 *a* and *b*. Mesenchyme cells and corpuscles of the control kept in filtered water.

FIG. 16. Epithelial cells of intestine of juv. *Q. pustulosa* after remaining in fat solution 24 hours.

FIG. 17. Portion of foot of same individual as in Fig. 16.

FIGS. 18 AND 19. Gill filaments of same individual as in Fig. 16.

FIG. 20. Portion of side of mantle next to body of same individual as in Fig. 16.

FIG. 21. *a*. Epithelial cells of that portion of mantle immersed in fat solution 22 hours. The mouth of this mussel was kept above the solution. *b*. Epithelial cells of corresponding part of mantle of mussel used as control for Fig. 21 *a*.

Fig. 16.

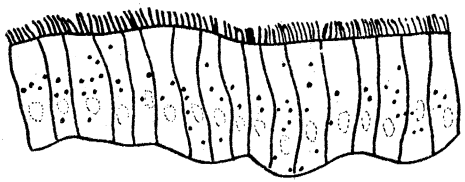


Fig. 17.

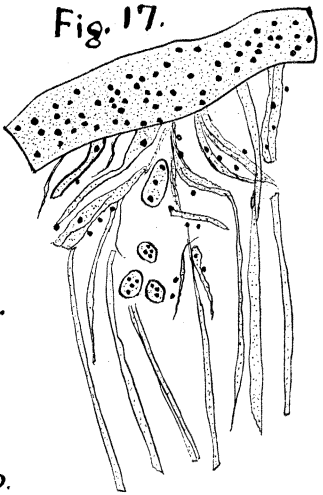


Fig. 12 a.

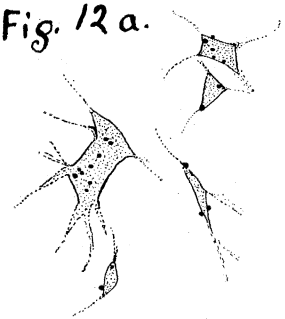


Fig. 12 b.

Fig. 13 b.

Fig. 21 a.

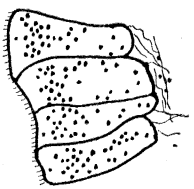


Fig. 18.

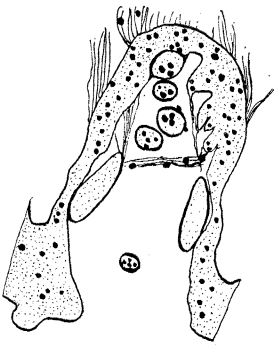


Fig. 13 a.

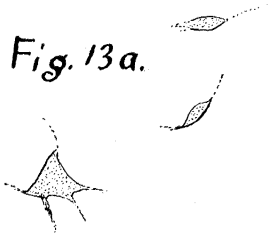


Fig. 21 b.

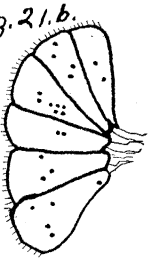


Fig. 19.

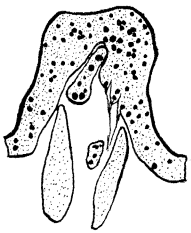


Fig. 20